

membrane. We found that isolated presynaptic varicosities are maintained and stable for at least two weeks allowing transfection and expression of fluorescent probes such as fusion constructs of the pH sensitive pHluorin protein. pHluorin is quenched at the acidic pH within vesicles and becomes fully fluorescent upon fusion. Neurons transfected with synaptophysin-pHluorin could be imaged after ten to 14 days in vitro by total internal reflection fluorescence microscopy (TIRFM). Where varicosities were formed on the functionalized micropatterns, we observed single fusion events, visible as diffraction-limited spots, on stimulation with single action potentials.

Thus, this novel purely presynaptic preparation allows us to monitor the dynamics of presynaptic exo- and endocytosis in unprecedented detail.

#### 517-Pos Board B297

##### Synaptotagmin-1 and Synaptotagmin-7 Differ in their Stimulus and $\text{Ca}^{2+}$ -Dependence of Activation

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The secretory response of adrenal chromaffin cells varies in accordance with the stimulus applied. Mild stimulation favors limited release of granule cargo through a narrow fusion pore while strong stimulation leads to greater release through wide pore expansion. The trigger for release in these cells is a rise in intracellular  $\text{Ca}^{2+}$  through voltage-gated channels. The level of intracellular  $\text{Ca}^{2+}$  accumulation varies directly with the strength of depolarization.  $\text{Ca}^{2+}$  triggered exocytosis in chromaffin cells also requires the protein synaptotagmin (Syt) as a sensor. The two isoforms expressed in these cells - synaptotagmin-1 (Syt-1) and synaptotagmin-7 (Syt-7) - have distinct  $\text{Ca}^{2+}$  affinities, are usually sorted to separate granules, and favor fundamentally different modes of exocytosis. Therefore, we wanted to determine whether changes in stimulation intensity drive release by selectively, or differentially, activating granules with Syt-1 or Syt-7. To address this question, we depolarized cells with varying levels of extracellular KCl. We found that while 56 mM KCl equally drove fusion of Syt-1 and Syt-7 granules, 10 and 25 mM KCl were far more effective at driving fusion of Syt-7 granules. To directly investigate  $\text{Ca}^{2+}$ -dependence of Syt isoform fusion, we perfused permeabilized cells with  $\text{Ca}^{2+}$  buffered to different concentrations with EGTA. Both Syt granule populations fused effectively at highest concentrations of  $\text{Ca}^{2+}$  (30  $\mu\text{M}$  and 100  $\mu\text{M}$ ), although there were small differences in their overall rate constants for activation. A greater fraction of docked Syt-7 granules fuse with significantly faster kinetics at a lower  $\text{Ca}^{2+}$  concentration (10  $\mu\text{M}$ ). Our results demonstrate chromaffin cells exploit Syt isoform diversity to modulate the secretory response. A significant consequence of synaptotagmin isoform segregation is that individual granules are endowed with unique  $\text{Ca}^{2+}$ -sensitivities, allowing rapid and local control of the fusion mode based on local  $\text{Ca}^{2+}$  levels.

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##### The Identification of VAMPs in B-Lymphocytes

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VAMPs (vesicle associated membrane proteins) are vital components for vesicle transport within the cell and exocytosis of materials from the cell. VAMP 1 and 2 are crucial for exocytosis of neurotransmitter while VAMP 3 is more ubiquitous in expression and function and VAMPs 7 and 8, which were once deemed to be endocytosis mediators, are now known to be involved in exocytosis in non-neuronal cells such as pancreatic acinar cells, mast cells and neutrophils. White blood cells other than mast cells and neutrophils have also been examined for VAMP content. Human B-lymphocytes have been found to display VAMP 2 and 3. Our laboratory has determined that VAMP 2 co-localized with immunoglobulin in Human RPMI 1788 cells. This finding was supported by the co-localization of VAMP 2 with the vesicular fraction of rabbit plasmacytoma-like 240 E cells as well. VAMP 3, which was previously identified in human cell lines, was found primarily associated with the plasma membrane fraction in 240 E cells. Since VAMP 7 has been found to be present in other white blood cell lines, we tested 240 E cells for the presence of VAMP 7. Our data confirmed the presence of VAMP 7 via whole cell fluorescence; however, it could not be verified by immunoblot techniques. We conclude that multiple VAMPs are present in plasma cells. VAMP 2 is most likely associated with exocytosis of antibody. VAMP 3 is also most likely involved with a mechanism at the plasma membrane and VAMP 7 may be present in the cell at low quantities with a role in the cell that has yet to be determined.

#### 519-Pos Board B299

##### Complexin: Masterpiece in Vesicle Cycling and Release

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Synaptic vesicle pools and cycling are crucial for neurotransmitter release and short-term plasticity. At the last step of vesicle fusion, the SNARE-complex binding protein Complexin clamps spontaneous fusion and promotes calcium-triggered release. In addition, the availability of the releasable vesicle pool is thought to be modulated by Complexin. However, the role of Complexin in vesicle cycling and release is still under investigation. Here we scrutinize the regulatory function of Complexin in the releasable pools and cycling at *Drosophila* neuromuscular junction. Nerve-evoked synchronous/asynchronous release is enhanced by Complexin and calcium. In addition, Complexin reduces the strontium evoked release sensitivity. The delayed asynchronous release induced by high-frequency nerve-stimulation is suppressed by Complexin and EGTA-AM. Moreover, delayed release is prolonged and early activated in Complexin null. These results indicate that Complexin suppress the asynchronous release activation either by strontium or by tetanic stimulation. Desensitization experiments discard the post synaptic receptor saturation during delayed release. Deconvolution analysis during trains of stimulation indicates that the synchronous, asynchronous and delayed releases are bidirectionally regulated by complexin. The time-course for depletion and the fluorescent dye unloading are consistent with reduced vesicle priming in Complexin null.

#### 520-Pos Board B300

##### Environmental Perturbations that Cause Structural Changes in the SNARE Protein SNAP-25

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The SNARE protein SNAP-25 plays a critical role in neuronal exocytosis by providing 2 of the 4 helical regions (SNARE domains) that form a coiled-coil quaternary structure. SNAP-25B contains four cysteines, one tryptophan and one tyrosine all in the linker region. Previously using molecular dynamics simulations, we showed that cysteine oxidation (disulfide bond formation) destabilizes the SNARE complex (Bock et al., 2010. Biophysical. J. 99:1221-1230). To test this prediction in vitro, we used Circular Dichroism and Fluorescence Spectroscopy to measure structural changes in SNAP-25B as a function of pH.

Using Circular Dichroism to measure the melting of SNARE domains, we show that oxidation destabilizes both the SNARE complex and SNAP-25B. A similar loss in helical structure is observed with an increase in pH. Using the fluorescent signal from tryptophan and tyrosine, an increase in pH changes the peak emission with a pK of ~8.5, similar to the expected pKa for the sulfhydryl of cysteine. Replacing all cysteines with serines prevents this shift. These data support the hypothesis that cysteine modification significantly alters SNAP-25 structure, possibly affecting exocytosis.

In vivo, cysteines are the sites of various posttranslational modifications, such as palmitoylation and oxidation. Palmitoylation anchors SNAP-25 to the membrane (and determines its distribution within the cell), while the level of oxidation may regulate neurotransmitter release.

Using an in vitro assay developed in our lab, we have measured the extent of non-enzymatic palmitoylation of SNAP-25B. Only 3 of the 4 cysteines are palmitoylated; the process is cooperative with a Hill Coefficient >2. However, all four cysteines are easily oxidized with a lower Hill coefficient. Through additional experimentation we hope to determine how oxidation and palmitoylation alter SNARE complex formation to modulate neurotransmitter release.

#### 521-Pos Board B301

##### Granuphilin C2A Domain as a Coincidence Detector for Phosphatidylserine and Phosphoinositides

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Granuphilin is a membrane binding protein responsible, in part, for docking insulin secretory vesicles to the plasma membrane. Granuphilin, also known as synaptotagmin like protein 4 (Slp4), contains tandem C2 domains. Unlike synaptotagmin, however, the granuphilin C2A domain binds strongly to plasma membrane lipids independent of intracellular  $\text{Ca}^{2+}$ . Despite the strong binding,